Interactions between Puumala hantavirus and its host, the bank vole, in the boreal zone

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Nothing is exactly as it seems,
nor is it otherwise.

– Alan Watts

Contents

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals. Articles I and II are reprinted with permission from their copyright holders.

I Kallio, E.R., Voutilainen, L., Vapalahti, O., Vaheri, A., Henttonen, H., Koskela, E., & Mappes, T. (2007). Endemic hantavirus infection impairs the winter survival of its rodent host. *Ecology* 88, 1911–1916. doi:10.1890/06-1620.1

II Voutilainen, L., Savola, S., Kallio, E.R., Laakkonen, J., Vaheri, A., Vapalahti, O. & Henttonen, H. (2012). Environmental change and disease dynamics: effects of intensive forest management on Puumala hantavirus infection in boreal bank vole populations. *PLoS ONE* 7, e39452. doi:10.1371/journal.pone.0039452

III Voutilainen, L., Sironen, T., Tonteri, E., Tuiskunen, A., Razzauti, M., Karlsson, M., Wahlström, M., Niemimaa, J., Henttonen, H. & Lundkvist, Å. Life-long shedding and chronic viremia of Puumala hantavirus in wild bank voles (*Myodes glareolus*). *Manuscript*.

IV Voutilainen, L., Kallio, E.R., Niemimaa, J., Vapalahti, O. & Henttonen, H. Temporal dynamics and demographic associations of Puumala hantavirus infection in cyclic populations of bank voles. *Manuscript*.

The author's contribution:

I Took part in the trapping of bank voles, the laboratory and statistical analyses and manuscript preparation. The article was included in Eva R. Kallio's doctoral thesis as a manuscript.

II Performed a part of the laboratory analyses, all statistical analyses and wrote the manuscript. The article will also be included in Sakeri Savola's doctoral thesis.

III Formulated the original idea, collected the samples in the field, performed all statistical analyses, and wrote the manuscript.

IV Performed most of the field collection and laboratory analyses, carried out the statistical analyses and wrote the manuscript.

Abstract

Among rodent-borne pathogens, hantaviruses are one of the most important groups concerning human health and economy. Understanding the interactions between hantaviruses and their reservoir host species is crucial for prediction and prevention of human epidemics. In this thesis, I studied the interactions between Puumala hantavirus (PUUV) and its host, the bank vole (*Myodes glareolus*). The study was conducted in the boreal zone of Europe where human incidence of nephropathia epidemica (NE, a mild form of haemorrhagic fever with renal syndrome) caused by PUUV is the highest.

Endemic pathogens, such as hantaviruses, have been hypothesized to cause no apparent symptoms or fitness costs to their hosts. However, we found PUUV infection to decrease the over-winter survival of wild bank voles. We also found wild bank voles to shed PUUV via urine, faeces, and saliva throughout their life span without any remarkable decline, in contrast to earlier results from laboratory-reared rodent hosts.

For the first time, dynamics of PUUV infection were studied during winter, when the majority of NE cases occur in the boreal zone. We found PUUV-infected bank voles to be most abundant in the winters of increase and peak years of the 3-year density cycle. In bank voles, the prevalence of PUUV infection showed a regular, seasonal fluctuation, which resulted from seasonal population turnover and the positive correlation between age and the likelihood of being PUUV infected. However, despite its regular fluctuation, PUUV prevalence in voles is not a good predictor of human NE risk since the periods of high prevalence coincided with low NE incidence in humans.

Aggression has been suggested as the key driver for other hantaviruses in their host species, but the rate of PUUV transmission in bank voles was higher outside the breeding season, when bank voles do not show aggressive behaviour, than during the breeding season. The high rate of transmission

outside the breeding season may be attributed to subnivean conditions that promote virus stability, lower immune response during cold conditions, and high host density in fall. We also found evidence for the "dilution effect" hypothesis, which assumes non-host species to reduce virus transmission between hosts: the prevalence of PUUV was low in bank voles when other small mammals were abundant.

Male sex bias in infection is a general phenomenon that has also been observed in several hantavirus-host systems. We found a male bias in PUUV infection only in overwintered, breeding bank voles, whereas a female bias was seen in summer-born breeding animals. In non-breeding animals, no sex differences existed. Therefore, the effects of host sex in hantavirus transmission may be more complex than previously thought.

Forest habitats disturbed by intensive forest management were associated with a higher likelihood of PUUV infection in bank voles. This finding could be explained by the poorer quality of these habitats, leading to lower condition and higher susceptibility, and also by more favourable environmental conditions for virus survival outside the host. Despite the higher infection prevalence in voles, the total number of PUUV-infected bank voles was 46-64% lower in young, intensively managed than in undisturbed, old forests. Thus, environmental change *per se* does not automatically lead to relative success of species that serve as reservoirs for zoonotic pathogens, and thereby, to increased human disease risk.

The results of this thesis encourage further studies of host-pathogen interactions in natural conditions, and in different host-hantavirus systems. They also provide a framework for risk models aiming at reduction of human hantavirus infections.

Tiivistelmä

Eläimistä ihmisiin tarttuvat, ns. zoonoottiset taudit ovat maailmanlaajuisesti suurimpia ihmisten terveyteen kohdistuvia uhkia. Villijyrsijät levittävät useita zoonooseja, joista merkittävimpien joukossa ovat hantavirukset. Hantaviruksiin kuuluvan Puumala-viruksen (PUUV) aiheuttama myyräkuume on Suomessa ja muualla Pohjois-Euroopassa yleinen zoonoosi, jota levittää koko Euroopassa yleinen metsämyyrä (*Myodes glareolus*). Väitöskirjassani tutkin Puumala-viruksen ja metsämyyrän vuorovaikutussuhdetta, jonka ymmärtäminen edesauttaa myyräkuume-epidemioiden ennustamista ja niihin varautumista.

Hantavirusten ei ole aiemmin havaittu aiheuttavan isäntäjyrsijöilleen haittaa tai näkyviä oireita. Tutkimuksessani Puumala-virustartunta heikensi metsämyyrien selviytymistä talven yli. Luonnonvaraiset metsämyyrät erittivät Puumala-virusta koko elinikänsä ilman merkittävää muutosta, kun taas useissa laboratoriokokeissa jyrsijöiden viruseritys on ollut runsasta vain tartunnan alkuviikkoina.

Vaikka myyräkuumetta esiintyy eniten talvisin, Puumala-viruksen esiintymistä metsämyyrissä ei ole talvisaikaan aiemmin tutkittu. Puumala-virusta kantavia myyriä oli luonnossa runsaimmin kolmevuotisen myyrien tiheyssyklin nousu- ja huippuvuosien talvina. Virusta kantavien myyrien suhteellinen osuus oli suurimmillaan aina keväisin, jolloin myyräkanta oli alhainen ja ihmistapauksia esiintyi yleensä vähän – tästä syystä virusta kantavien myyrien osuus ei ole myyräkuumeriskin arviointiin sopiva mittari.

Useiden hantavirusten on arveltu leviävän etupäässä isäntäjyrsijöiden välisissä yhteenotoissa. Tutkimuksessani havaitsin tätä vastoin vähemmän Puumala-virustartuntoja metsämyyrien lisääntymisaikana, jolloin myyrät käyttäytyvät aggressiivisesti, kuin muina vuodenaikoina. Tehokkaampi leviäminen talven aikana voi johtua muun muassa viruksen paremmasta säilymisestä lumenalaisissa, kylmissä ja kosteissa oloissa.

Lajiyhteisön monimuotoisuuden on esitetty vähentävän tautien leviämistä isäntälajissaan niin sanotun ”laimennusvaikutuksen” kautta, mutta ilmiöstä on vain vähän havaintoja. Tutkimuksessani muiden pikkunisäkkäiden, kuten peltomyyrien ja päästäisten runsas määrä vähensi Puumala-virusinfektion yleisyyttä metsämyyrissä ”laimennusvaikutuksen” mukaisesti.

Ympäristömuutosten on arveltu maailmanlaajuisesti lisäävän zoonoottisten tautien leviämisriskiä ihmisiin. Tutkimuksessani havaitsin, että nuorissa, metsätalouden alaisissa metsissä oli noin puolet vähemmän Puumala-virusta kantavia metsämyyriä kuin vanhoissa, lähes luonnontilaisissa metsissä. Siten ympäristömuutosten vaikutukset tautiriskiin riippuvat niiden aiheuttamista lajyhteisömuutoksista.

Väitöskirjatyöni tulosten perusteella on tärkeää tutkia zoonoottisia taudinaiheuttajia laboratoriokokeiden lisäksi myös niiden luonnollisessa ympäristössä. Työni tuloksia voidaan hyödyntää Puumala-viruksen leviämistä ja myyräkuumeriskiä ennustavissa matemaattisissa malleissa, joiden pyrkimyksenä on myyräkuume- ja muiden hantavirustartuntatapausten vähentäminen tulevaisuudessa.

1. Introduction

The evolutionary histories of most human infectious diseases point towards origins in non-human animals (Taylor *et al.* 2001, Woolhouse & Gowtage-Sequeria 2005). Recent outbreaks of diseases like SARS and highly pathogenic bird and swine influenzas have shown emergence of zoonoses, i.e. diseases that transmit from vertebrate animals to humans, to occur in our times as well. Alterations in the environment, such as in climate and habitats, as well as socioeconomical changes facilitate human contact with novel pathogens, and as such, have and presumably will trigger events of disease emergence (Morse 1995, Jones *et al.* 2008, Daszak *et al.* 2013).

Rodents represent more than 40% of all known mammal species (Wilson & Reeder 2005) and many of them reside in close contact with humans either as agricultural pests or household commensals. The breeding capacity of rodents is high, often resulting in population eruptions when conditions are favourable. These characteristics make rodents important carriers of zoonotic diseases. Rodents also serve as sentinels for diseases that are transmitted to humans or domestic animals from a vector species, such as ticks, mites, and fleas. The role of rodents is crucial in the circulation of many important diseases in terms of human health and economy, such as hantaviral and arenaviral diseases, leptospirosis, plaque, Lyme disease and Crimean-Congo haemorrhagic fever (Meerburg *et al.* 2009).

1.1 Hantaviruses

1.1.1 Evolutionary history and human epidemiology

Twenty-five years after an epidemic of haemorrhagic fever among military troops in Korea in the 1950s (Earle 1954), the causative agent, named Hantaan virus, was isolated both from a diseased patient and from the striped field mouse (*Apodemus agrarius*, Lee *et al.* 1978). Numerous similar viruses were thereafter discovered from different rodent species and assigned to a new genus *hantavirus* among the family *Bunyaviridae* (Elliot 1990). Remarkable parallel divergence of host and virus species indicated

co-evolution of the two (Plyusnin *et al.* 1996, Yates *et al.* 2002). Recently, new insights to the evolution of hantaviruses have been revealed by the discovery of several hantaviruses specific to shrew, mole and bat hosts (Henttonen *et al.* 2008, Guo *et al.* 2013, Figure 1).

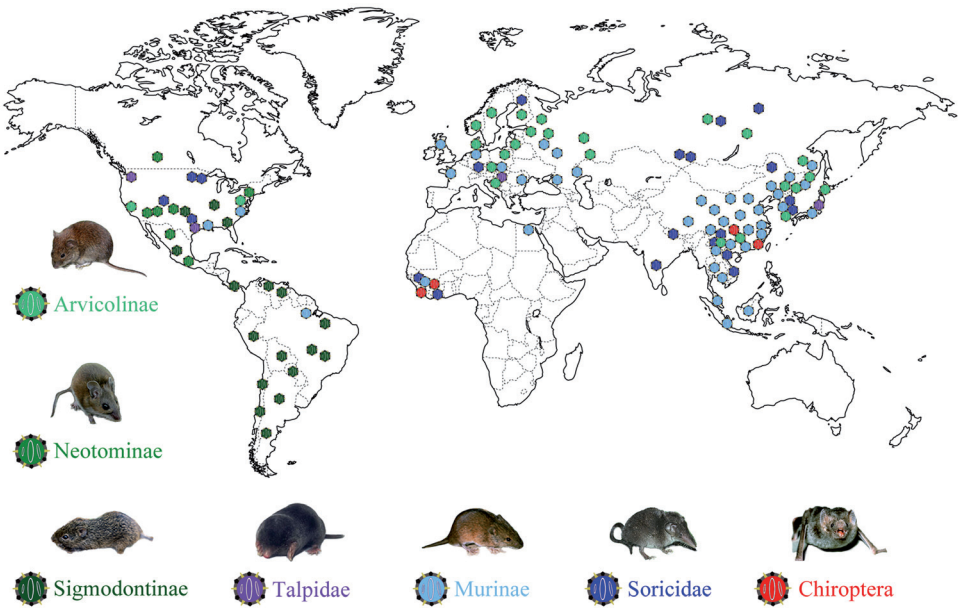

Figure 1. The distribution of known hantaviruses by associated mammalian host group. Reprinted from (Guo et al. 2013) with permission.

Humans are incidental hosts for hantaviruses and acquire infection through aerosolized excretions of rodents (Vapalahti *et al.* 2010). Old World rats and mice (family Muridae, subfamily Murinae) carry hantaviruses that cause haemorrhagic fever with renal syndrome (HFRS) in humans, leading up to 12% case fatality rate (CFR, Jonsson *et al.* 2010 and references therein, Figure 2). New World hantaviruses are carried by rodents of subfamilies Neotominae and Sigmodontinae (family Cricetidae) and cause hantavirus pulmonary syndrome (HPS) with up to 60% CFR (Jonsson *et al.* 2010). Puumala virus (PUUV, Brummer-Korvenkontio *et al.* 1980) is carried by bank voles (*Myodes glareolus*, family Cricetidae, subfamily Arvicolinae) nearly throughout the distribution range of the species in Europe and Russia

(Olsson *et al.* 2010). In humans, PUUV causes nephropathia epidemica (NE), a mild form of HFRS with a low CFR (<0.1%). The disease accounts for the most of hantavirus infections in Europe and is by far most common in northern Europe (Figure 2, Heyman *et al.* 2008).

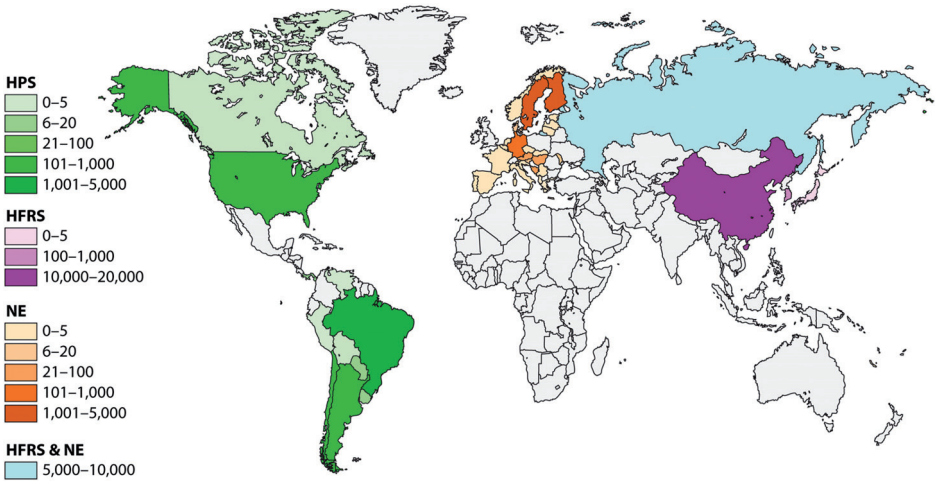

Figure 2. The worldwide distribution of human disease (yearly incidence per country) due to hantavirus infections (Jonsson *et al.* 2010), reprinted with permission from American Society for Microbiology). HPS=hantavirus pulmonary syndrome, HFRS=haemorrhagic fever with renal syndrome, NE=nephropathia epidemica.

Currently, no vaccine against HFRS, HPS, or NE is available in Europe or the Americas (Jonsson *et al.* 2010). Hence, the remaining means to control human epidemics of hantavirus infections are reducing rodent-human contact and predicting periods of high disease risk. In general, human epidemics follow high densities of host rodents (Niklasson *et al.* 1995, Mills & Childs 1998, Olsson *et al.* 2003, Hu *et al.* 2007, Kallio *et al.* 2009). In the boreal zone, highest rates of NE cases occur during late fall and winter (Brummer-Korvenkontio *et al.* 1999, Olsson *et al.* 2003 and 2009, Kallio *et al.* 2009) Makary *et al.* 2010) whereas in the temperate region, NE incidence peaks occur in summer (Heyman *et al.* 2001, Tersago *et al.* 2011).

1.1.2 Interactions between hantaviruses and rodent hosts

Assumedly, the ultimate driver of human hantavirus disease incidence is the abundance of infected hosts, which is a product of host abundance and virus prevalence (Davis *et al.* 2005). Interactions between the host and the virus, in turn, shape the transmission dynamics and thus the prevalence of infection in the host population.

Hantavirus transmission between rodent hosts occurs horizontally through inhalation of infectious excreta or in direct contact (Lee *et al.* 1981, Yanagihara *et al.* 1985), and viral particles remain infectious outside the host for several weeks (Kallio *et al.* 2006a). Once infected, hosts develop a specific antibody response within 30 days after infection (Lee *et al.* 1981, Yanagihara *et al.* 1985, Botten *et al.* 2000). Infectious virus can be found in the tissues and excreta for several months, however most laboratory experiments suggest only a brief viremia and the viral shedding to decrease by time (Lee *et al.* 1981, Yanagihara *et al.* 1985, Gavrilovskaya *et al.* 1990, Fulhorst *et al.* 2002, Botten *et al.* 2003, Hardestam *et al.* 2008). The infection causes no apparent symptoms (Verhagen *et al.* 1986, Gavrilovskaya *et al.* 1990, Hutchinson *et al.* 1998), although some changes in tissue morphology have been reported (Gavrilovskaya *et al.* 1983, Netski *et al.* 1999, Billings *et al.* 2010). Hantavirus-specific antibodies are transmitted from infected mothers to their pups, providing immunity for up to 80 days of age (Gavrilovskaya *et al.* 1990, Dohmae *et al.* 1993, Kallio *et al.* 2006b).

As the transmission of hantaviruses is horizontal, it is assumed density-dependent, i.e. to increase with host density due to the consequently increased contact rate (Mills *et al.* 1999). Recently, hantavirus transmission has also been suggested to depend on the species community; the "dilution effect" hypothesis originally formulated for vector-borne pathogens (Norman *et al.* 1999, Keesing *et al.* 2010) has been extended to directly transmitted hantaviruses, postulating that lower diversity in host community, which is often associated with disturbed habitats, increases the contact rate between host individuals and thus the hantavirus transmission rate (Keesing *et al.* 2010, Dearing & Dizney 2010).

1.2 The biology of the bank vole

The range of the bank vole reaches from the British Isles across most of continental Europe to Lake Baikal. The species inhabits all types of forest, but is also found in densely vegetated clearings, hedge networks and parks (Amori *et al.* 2012). In Finland, breeding is constrained to summer months, i.e. from May to September, during which time the females may produce up to four litters of 2–10 pups (Koivula *et al.* 2003). In temperate regions, the breeding season is longer and winter reproduction occurs occasionally (Pucek *et al.* 1993, Tkadlec & Zejda 1998). Breeding females are territorial, while males have larger home ranges overlapping those of females' (Koskela *et al.* 1997). In general, bank voles born in early summer mature at 3–4 weeks of age, and those born later in summer postpone their breeding until the next summer (Prévot-Julliard *et al.* 1999). During winter, bank voles show no territorial behaviour and are therefore more tolerant to conspecifics (Ylönen & Viitala 1985).

In the boreal Europe, bank vole populations fluctuate in regular, 3–5 year cycles that are assumed to be driven by specialist predators (Hanski & Henttonen 1996, Hanski *et al.* 2001, Sundell *et al.* 2004), whereas in the temperate zone, bank vole outbreaks result from climate-driven masting events, i.e. high production of oak acorns and beechnuts (Jensen 1982, Pucek *et al.* 1993, Piovesan & Adams 2001, Kelly *et al.* 2013)

2. The aims of the thesis

The aim of this thesis was to increase our understanding of epizootic processes of Puumala hantavirus (PUUV) in its host populations in the boreal zone, where human disease incidence is the highest. Specifically, this study entails the following (Figure 3):

- Effects of PUUV infection on bank vole survival in nature (I)
- PUUV shedding in natural settings (III)
- Effects of habitat and small mammal community on PUUV infection (II)
- Temporal dynamics of infection in cyclic bank vole populations (II & IV)
- Demographic associations of PUUV infection in bank voles (II & IV)

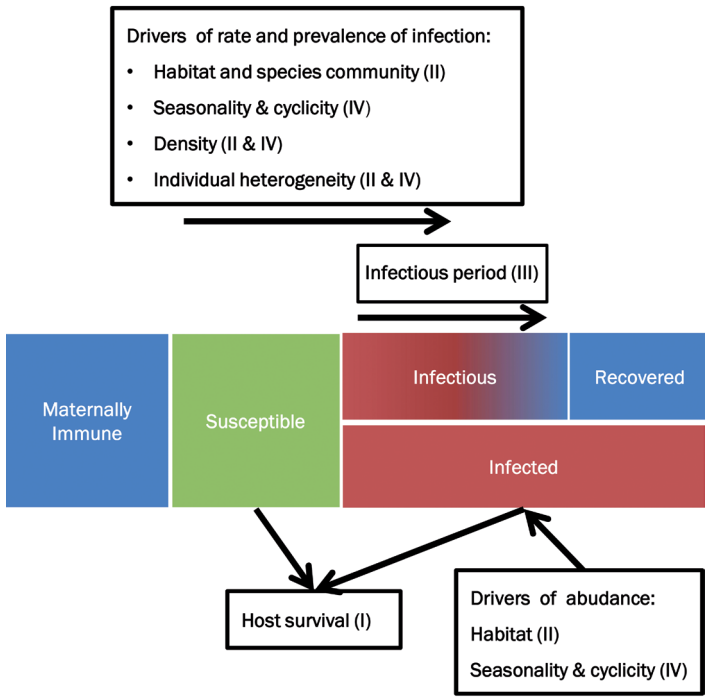

Figure 3. Processes and attributes of hantavirus transmission studied in this thesis. The coloured boxes indicate the compartments of the classical SIR (Susceptible, Infected, Recovered) model modified for hantavirus transmission.

3. Materials and methods

3.1 Study sites and trapping design

All study sites were located in boreal forests, a natural habitat of bank voles (Figure 4A-B). Studies I, III, and IV were conducted in the municipality of Konnevesi, central Finland (62°37' N, 26°20' E), and study II in the municipalities of Taivalkoski and Pudasjärvi, northern Finland (65° N, 28° E). The annual incidence of nephropathia epidemica in the corresponding health care districts (Central Finland and Northern Bothnia) averaged 42.5 and 70.9 cases per 100 000 inhabitants, respectively, in 1995–2008 (Makary *et al.* 2010), and therefore PUUV was regarded endemic in local bank vole populations.

Study I was conducted on 22 islands of size 0.24–3.20 ha scattered on an area of 72 km^2 on Lake Konnevesi (Figure 4K) during three consecutive winters (trapping periods every October and May, years 2002-2005). Study IV was conducted on one 5.8 ha capture-mark-recapture grid ("core grid") and fourteen 0.09 ha removal trapping grids ("satellite grids") on an area of 25 km^2 on Konnevesi mainland (Figure 4K) between April 2002 and May 2009. On the core grid, the interval between two trapping periods ranged from 17 to 166 days (median 37 days) and trappings were conducted throughout the year. On satellite grids, trappings were performed every May, July, and October. Bank voles trapped on the core grid between January 2008 and May 2009 were used in (III). Study II was conducted every June and September in 2007–2010 on 200 15 m*15 m small quadrats (removal trapping grids) located on 40 forest compartments distributed on an area of 875 km^2 (Figure 4T). The selected forest compartments represented four age classes; i) non-managed forests older than 100 years and managed forests with ii) 25–30, iii) 10–15 and iv) 4–8 years from plantation after clear cutting. In (II-IV), the interval between two trap stations was 15 m and in (I), 25 traps were set per hectare in approximately 20 m intervals. In (IV), three snap-traps were set on each trap station, whereas in other studies, only one trap was used.

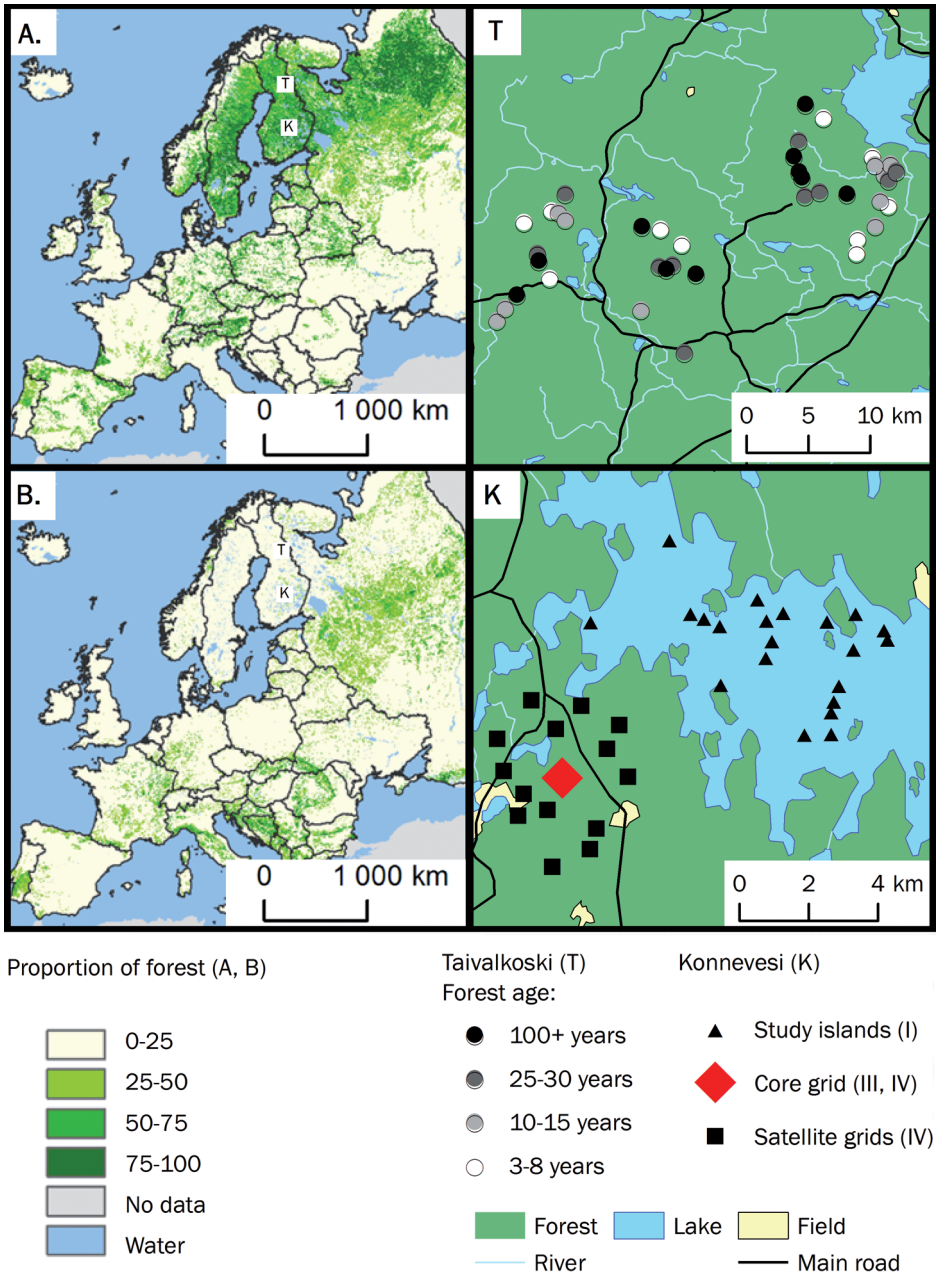

Figure 4. Land cover percentages of coniferous (A) and deciduous (B) forests in Europe and the study designs in Taivalkoski (T) and Konnevesi (K). The locations of Taivalkoski and Konnevesi are marked in A and B.

3.2 Trapping and sampling procedures

For individual monitoring in capture-mark-recapture studies (I, III, IV), bank voles were captured using Ugglan Special live-traps (Grahnab, Sweden). Captured animals were marked individually using either toe clipping (I) or subcutaneous passive inductive transponder (PIT) tags (Trovan, U.K., III and IV). Before releasing the bank voles back to the site of capture, their sex, breeding status, body mass, age (year-born/overwintered) were recorded and a blood sample was taken via the retro-orbital sinus. In (III), also faecal, urine, and saliva samples (the latter using flocked swabs, Copan, U.S.) were taken.

Removal trapping was conducted in (II) and (IV). All trapping in (II) and 3/21 of the trapping periods on the "satellite grids" in (IV) were conducted using standard mouse snap-traps baited with rye bread. The animals were frozen in –20°C and dissected later. During dissection, sex, breeding status, body mass and age were recorded and the heart was stored in 200 µL phosphate-buffered saline and frozen to be later used for determination of PUUV antibodies. In the remaining 18 trapping periods on the satellite grids in (IV), Ugglan Special live-traps were used. All trapping and animal handling procedures were in concordance with the Finnish Act on Animal Experimentation (62/2006).

3.3 Molecular analyses and determination of PUUV infection

Puumala virus infection in bank voles was determined from blood and heart samples using immunofluorescent antibody test (IFAT) against PUUV antibodies as described earlier (Kallio-Kokko *et al.* 2006). As hantaviruses cause a chronic infection in their rodent hosts (Meyer & Schmaljohn 2000), the presence of PUUV antibodies could be taken as an indicator of acquired infection. However, in young bank voles, detected antibodies could have been transferred from their mother during gestation and lactation (Dohmae *et al.* 1993, Kallio *et al.* 2006b). Therefore, generalized additive mixed model

(GAMM) predictions based on the J-shaped curve between body mass and the probability of being antibody positive (II, see Kallio *et al.* 2010) or on the body masses of individuals with a known infection history (IV) were used to distinguish between maternal antibodies and an acquired infection. In the other two studies this distinction was not necessary, as the study animals were tested for PUUV antibodies after the end of the breeding season (I) when maternal antibodies have disappeared (see Kallio *et al.* 2010 and IV), or since the selection of studied individuals was based on observed PUUV seroconversion (III).

A reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) method specific to the Small genome segment of the local PUUV strain (Razzauti *et al.* 2008) was used to quantify the amount of PUUV in bank vole blood, saliva, faeces, and urine (III).

3.4 Statistical analyses

In all four studies, mixed effects models (Pinheiro & Bates 2000, Paterson & Lello 2003, Bolker *et al.* 2009) were used for data analyses. Using this approach, the confounding effects of different explanatory variables, as well as pseudoreplication due to repeated measurements from a single animal, time point, or a study site could be accounted for. Where appropriate, information theoretic selection of models (Burnham & Anderson 2000, Johnson & Omland 2004) was used to find the most parsimonious models, i.e., combinations of predictor variables that best explained the observed variation in the response variables.

4. Results and discussion

4.1 Consequences of PUUV infection in wild bank voles

We found PUUV infection to decrease the probability of over-winter survival of bank voles in island populations (I). This detrimental effect was seen on both the individual and population levels when potentially confounding factors (age, body mass, sex; population size and density, island size) were controlled for. This was the first time that hantaviruses have been shown to impair the host's survival, and recently, our findings have been supported by similar findings on PUUV in bank voles in the temperate zone (Tersago *et al.* 2012) and on Sin Nombre hantavirus in deer mice (*Peromyscus maniculatus*) in North America (Luis *et al.* 2012, but see Previtali *et al.* 2010). Our results raise the question whether the deleterious effects of PUUV could actually contribute to the cyclic fluctuations of vole populations in northern Europe — this, however, is not likely as PUUV is specific to bank voles, whereas the cyclic fluctuations occur synchronously in all arvicoline species in the region (Hansson & Henttonen 1988, Huitu *et al.* 2004).

We demonstrated that in free-ranging bank voles, the shedding of PUUV via saliva, urine, and faeces continues beyond the average life span of the host (Innes & Millar 1994), i.e., up to 8 months after acquiring PUUV infection (II). Shedding was most intensive during the first month after seroconversion to PUUV, but in contrast to studies performed on laboratory-reared hosts, neither the proportion of animals shedding the virus nor the quantity of shed virus declined drastically by time from infection. The virus was also present in blood for several months, which is in line with the high proportions of viremic individuals observed among wild hosts of other hantaviruses (Kuenzi *et al.* 2005, McIntyre *et al.* 2005, Safronetz *et al.* 2008). Persistent shedding by chronically infected hosts may be an important mechanism for PUUV to survive through host population bottlenecks. Such a mechanism is a pre-requisite for the survival of a directly transmitted pathogen where vectors do not contribute to long-term continuity of transmission.

The results of these two studies show that the behaviour of endemic pathogens in their free-ranging hosts may remarkably differ from that observed in laboratory studies both in terms of survival consequences and propagation. These differences may arise from various reasons that are not mutually exclusive – either methodological issues, such as lowered infectiousness of inoculated virus strain (Lundkvist *et al.* 1997), non-natural infection doses (Hardestam *et al.* 2008) – or limited resource availability in natural vs. laboratory conditions. Trade-offs between immune defence, survival and breeding (Martin *et al.* 2008, Mills *et al.* 2010) apparently translate as a longer infectious period and lowered survival in free-ranging hantavirus-infected rodents. Such effects may only manifest under harsh conditions, as our studies concentrated in the winter months. From another view, the outcomes of pathogen infections are a result of a complex interplay between the tolerance and resistance of host towards a pathogen, and on the other hand, the virulence of the pathogen (Råberg *et al.* 2007, Råberg *et al.* 2009 and Ayres & Schneider 2012). Thus it is possible that the individuals that survive PUUV infection over winter are the more tolerant and less resistant ones, which do not allocate their resources in "excess" immune defence and thus also shed the virus persistently. This mechanism could explain how a virus with a detrimental effect on host survival can persist over host population bottlenecks.

4.2 Factors influencing PUUV infection

4.2.1 Demographic associations

In host population, the prevalence of a chronic infection can be expected to increase with age as a result of accumulated time of exposure. This indeed was the case with PUUV infections, as we showed the infection prevalence to reach nearly 100% in bank voles that survived until the end of their second summer (IV), beyond which they rarely survived. The age-accumulated infection prevalence combined with the seasonal breeding resulted in high prevalence of PUUV infection in spring before the onset of breeding season, and low prevalence towards the end of the breeding season, when the young of the

year superseded the overwintered animals in the population. This "juvenile dilution effect" (Mills *et al.* 1999, Davis *et al.* 2005) has been assumed to underlie the seasonal fluctuation of hantavirus infection prevalence, which was seen here (II, IV) and in several other studies both in boreal (Niklasson *et al.* 1995, Olsson *et al.* 2002, Razzauti *et al.* 2008, Kallio *et al.* 2010) and in temperate populations of bank voles (Verhagen *et al.* 1986, Escutenaire *et al.* 2000), and in other hantavirus host species that show seasonal breeding (Boone *et al.* 1998, Mills *et al.* 1999, Douglass *et al.* 2001, Mills *et al.* 2007).

Male-biased prevalence or intensity of infection in mammal hosts has been reported for several parasites (Poulin 1996, Krasnov *et al.* 2012), and in the majority of studies on hantaviruses in their reservoir hosts, often being limited to reproductively active or older individuals (Mills *et al.* 1997, Douglass *et al.* 2001, Escutenaire *et al.* 2002, Olsson *et al.* 2002, McIntyre *et al.* 2005, Calisher *et al.* 2007, Kallio *et al.* 2010, Tersago *et al.* 2011). The phenomenon is assumed to result from larger home ranges, higher contact rates, or higher susceptibility of males. We demonstrated the same associations in cross-sectional data (II), but our mark-recapture study (IV) showed a more complex pattern; as in previous studies, a male bias was seen in overwintered, reproductive animals and no difference between sexes was seen in sexually inactive animals. However, young, breeding females were more likely to acquire infection and to be infected than breeding males or non-breeding animals of the same age. This may be due to their more frequent contact with old males, which most often were infected, or virus transmission in aggressive encounters with other breeding females during the territory establishment (Koskela *et al.* 1997, Kapusta *et al.* 2007 and 2012).

4.2.2 Population and community level determinants

The hypothesis of "dilution effect" on disease transmission by less or non-competent species has been widely studied among vector-borne diseases, and recently, also tested on hantaviruses (reviewed by Randolph & Dobson 2012). We found breeding bank voles to be less often PUUV infected when more individuals of other small mammal species (mostly *Microtus* spp. and *Sorex* shrews) were present (II). Concurrent host density, on the other hand,

was positively correlated with infection, but as it was controlled for in the analyses, it did not contribute to the observed "dilution effect" of non-hosts. The presence of *Microtus* spp. (Eccard & Ylönen 2002 and Eccard & Ylönen 2007) and shrews (Liesenjohann *et al.* 2011) has been shown to decrease the motility of bank vole females, and we assume this as the underlying mechanism – when these species are present, bank voles have less contacts to infectious conspecifics or environment, and thus lower probability of acquiring infection. Likewise, deer mice were less often infected with Sin Nombre virus at times when *Microtus* spp. were present (Carver *et al.* 2011). Bank voles in the young, intensively managed forests were more likely to be PUUV-infected than those in undisturbed, old forests (II), indicating that some component in the disturbed habitat itself that was not attributed to demographic factors, host density, or the small mammal community, resulted in higher infection risk. Undisturbed forests were preferred by bank voles, indicating they provided better resources than managed forests. Therefore, higher infection risk in disturbed habitat could result from compromised immune defence due to poorer condition in poorer habitats. It is also possible that the environmental conditions (high humidity and low UV radiation) in the forest class associated with highest probability of infection, i.e. the 25–30 year-old thickets where only a little sunlight was filtered to the forest floor, maintained higher loads of PUUV in the environment.

The transmission rate of PUUV was higher outside than during the breeding season (IV), suggesting that aggression is not an important route of PUUV transmission in boreal bank voles, although it has been emphasized in other rodent-hantavirus systems (Hinson *et al.* 2004, McIntyre *et al.* 2005, Calisher *et al.* 2007). Furthermore, in several years, transmission rate increased between August and October, coinciding with cessation of breeding, cooling temperature, and increasing density. As shown before (Kallio *et al.* 2010), maternal antibodies were more prevalent during peak years of density cycle as compared to increase years. The higher proportion of maternally immune individuals in the population may have delayed the transmission, leading to similar prevalences of PUUV infection in increase and peak years despite their 2-fold difference in maximum host densities.

4.3 Variation in the abundance of infected bank voles

The combined dynamics of the bank vole population and the transmission of PUUV resulted in very similar multiannual patterns in the course of two consecutive vole density cycles (IV). During these cycles, the density of infected bank voles remained higher than 50% of the maximum density for more than 15 months, i.e. from fall/winter of the increase year until winter/spring of the peak year, suggesting a high risk of human NE in the area. Indeed, the highest NE incidence in the same region was recorded in the winters of increase and peak density years (Kallio *et al.* 2009), when also the densities of infected animals were the highest. Interestingly, during the increase winter 2004-2005, when the total bank vole density reached 44% of that in the following peak winter 2005-2006, both the numbers of infected individuals and NE human incidence were higher (30% and 22%, respectively) during the increase than in peak winter. This result suggests that although human epidemics may well be predicted by the total host density (Kallio *et al.* 2009, Olsson *et al.* 2009), they are, nevertheless, driven by the abundance of infectious hosts which may vary irrespective of total host density.

Several studies have shown habitat disturbance to increase the prevalence of hantavirus infection in reservoir hosts or the incidence of human disease (Dearing & Dizney 2010 and references therein). By contrast, we found human-induced change in boreal forest environment, i.e., the intensive forest management to decrease the abundance of infected hosts by 46–64% (II). This finding together with results from other hantavirus-host systems (Polop *et al.* 2010) suggests that anthropogenic disturbance *per se* does not automatically lead to relative success of resilient species that often serve as competent reservoirs for zoonotic pathogens (Keesing *et al.* 2010) and thereby increase human disease risk. In our study, *Microtus* species, that have not been reported to carry pathogenic hantaviruses, are naturally associated with open habitats, and are competitively superior to bank voles, thrived in the most disturbed habitats whereas bank voles were most abundant in unmanaged, old forests (IV, see also Savola *et al.* 2013).

5. Conclusions and prospects

In this thesis, I studied the PUUV infection-induced mortality in wild bank voles, the length of shedding and viremia, individual and population-level factors associated with infection and spatiotemporal variation in the abundance of infected hosts in the boreal zone. I found that in natural conditions, infection by an endemic pathogen may affect the survival of the host and result in life-long infectious period – both of which are important attributes of disease dynamics. These findings should be taken into account in future modelling of hantavirus transmission.

Individual heterogeneities in susceptibility to infection appear to be more complex than previously thought, so that the presence and direction of sex bias in hantavirus infection appears to depend on the age and breeding status of the host. In contrast to other hantavirus-host systems, where the role of aggressive encounters in virus transmission has been emphasized, a higher rate of PUUV transmission between bank voles was seen outside the breeding season, indicating that the key routes of transmission may vary between hantavirus-host systems.

Within host-virus pairs, differences in host population dynamics between biomes and the resulting temporal patterns of infection may lead to differential human disease dynamics. Knowledge of biome-specific dynamics of diseases and host populations becomes particularly important in the face of the changing climate; in the temperate zone, the incidence of NE is suspected to increase due to more frequent masting events brought about by warming climate (Clement *et al.* 2009, Klempa 2009, Tersago *et al.* 2009, but see Kelly *et al.* 2013). In the most populated part of Finland, warming climate has been predicted to increase vole cyclicity (Korpela *et al.* 2013), which would presumably lead to pronounced epidemics of NE in the country.

My results encourage further studies of host-pathogen interactions in natural conditions, and in different host-hantavirus systems. Besides demographic characteristics, also immunogenetic properties of the host have been found important in mediating hantavirus transmission (Deter *et al.*

2008, Guivier *et al.* 2010). Recently, the role of interactions between different pathogens within the host have also been emphasized (Telfer *et al.* 2010, Tompkins *et al.* 2011), and future studies should aim at disentangling the relative importance of these factors in hantavirus transmission.

My thesis provides a solid framework for risk models aiming at public health measures to reduce human infections. This work adds to the growing body of research on the ecology of hantaviruses, which may serve as a basis for prediction and control tool for outbreaks of rodent-borne diseases yet to emerge.

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I finally did it!

Liisa Vanhatalo

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